FOUR-WEEK-LONG TABUN LOW-LEVEL EXPOSURE IN RATS

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ABSTRACT

Prolonged administration of sublethal doses of organophosphorus cholinesterase inhibitors results in adaptation to their toxicity. In order to investigate this phenomenon, we exposed rats to 0.2, 0.3 or 0.4 LD_{50} of tabun *sc* daily during four weeks. AChE activities in erythrocytes, diaphragm and brain were inhibited dose-dependently after days 7 and 14 of the study and started to recover thereafter, except after 0.4 LD_{50}. Tabun 0.3 LD_{50} decreased body weight gain, food and water consumption during the first two weeks of the study. Spontaneous locomotor activity was significantly increased in the same interval and decreased thereafter. These findings support the assumption that both the biochemical and receptor mechanisms are responsible for the occurrence of tolerance to tabun in rats.

INTRODUCTION

There is a plethora of the experimental and clinical studies dealing with the consequences of the poisonings with organophosphorus compounds (OPCs), including OP insecticides (OPIs) and nerve agents tabun, sarin, soman and VX. At the same time, the corresponding data on the effects of subacute low-level exposure to OPCs are rather scarce (1). The majority of the presently known publications on the subjects are four or more decades old and they are dealing with subacute poisoning with OPIs, such as parathion (2), octamethyl pyrophosphoramide (3), chlorthion, tetrapropyl dithionopyrophosphate, malathion (4), dipterex (5), systox (6) and di-syston (7). After proving that repeated administration of a meant-to-be-nerve agent diisopropylfluorophosphate (DFP) leads to development of tolerance (8), the first experimental study addressing the same issue in a similar manner, but employing real nerve agents instead of OPIs, was the one on tabun, sarin and soman subacute toxicity in rats (9). The reason for this disparity between the research in the OPI and nerve agent field was the fact that interest in the effects of OPCs after prolonged low-level exposure originated from the industrial hygiene. In fact, the stimulus for the investigations came from the notion that those individuals that are engaged in the manufacture or use of the OPIs could receive and survive repeated exposure to small doses. It was much later, when the

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military toxicologists started to share this initially purely civilian interest in the subacute toxicity of the specific OPCs, i.e. nerve agents (9-15).

Tabun was the first nerve agent to be synthesised by Dr. Gerhard Schrader of I.G. Farbenindustrie, on 23 December 1936 (16). The aim of this study was to try to shed some more light on the effects of the subacute effects of this potent irreversible inhibitor of acetylcholinesterase (AChE), when administered on a daily basis and to reveal the underlying mechanisms of tolerance to tabun.

METHODS

Male Wistar rats were used throughout the experiment. The median lethal dose (LD_{50}) of tabun was 150 μg/kg subcutaneously (*sc*). Animals were exposed to 0.2, 0.3 or 0.4 LD_{50} of tabun *sc* daily over a four-week period during which a number and time of fatalities was registered. Groups of control and tabun-poisoned animals (n=4) were sacrificed on days 7, 14, 21 and 28 in order to obtain tissue samples for biochemical analyses. For these purposes AChE activities in brain, diaphragm and erythrocytes were analysed spectrophotometrically (17). In the group of rats treated with 0.3 LD_{50} of tabun, body weight gain, food and water consumption (18) and spontaneous locomotor activity - or SLA (19) were monitored on a weekly basis.

RESULTS

All the rats treated on a daily basis with 0.2 LD_{50} of tabun survived four weeks. Cumulative mortality in the 0.3 LD_{50} group was 17% - the first animal died on the day 12 and the last one on the day 20 of the study. In the 0.4 LD_{50} group, a total of 88% died, between the treatment days 6 and 20 (Figure 1).

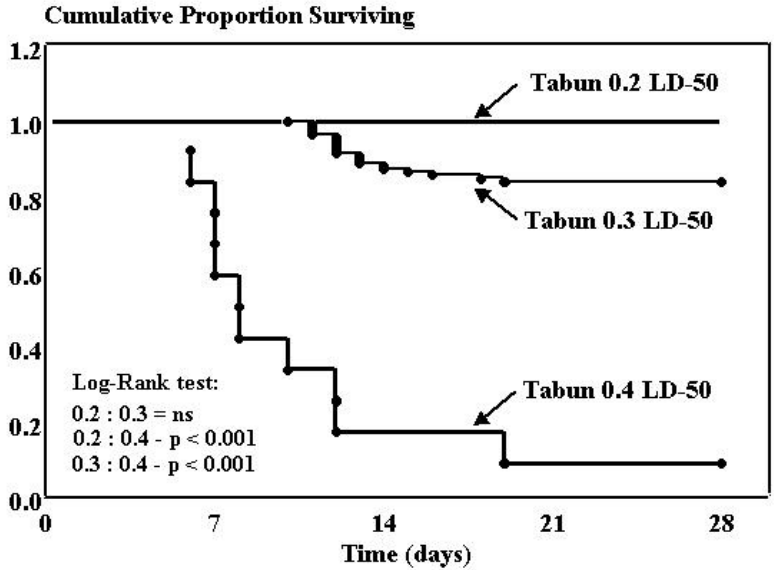
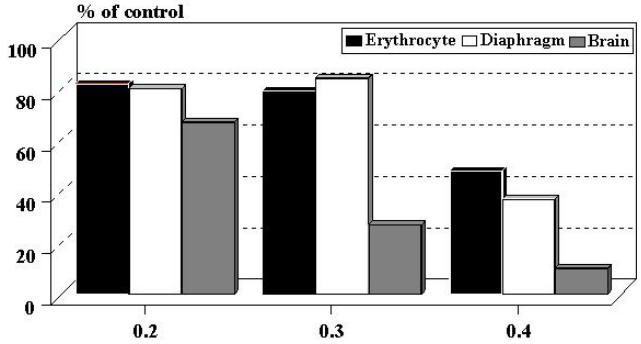


Figure 1. Cumulative mortality rates in rats treated with tabun 0.2, 0.3 or 0.4 LD_{50} daily.

AChE activities in all the tissues investigated and especially in the brain was dose-dependently inhibited with tabun. The sharpest decrease in the enzyme activities was found after days 7 and 14 of the study. In two lower dose groups gradual recovery of the enzyme activities was noticed during the last two weeks (Figure 2).

Week 1.



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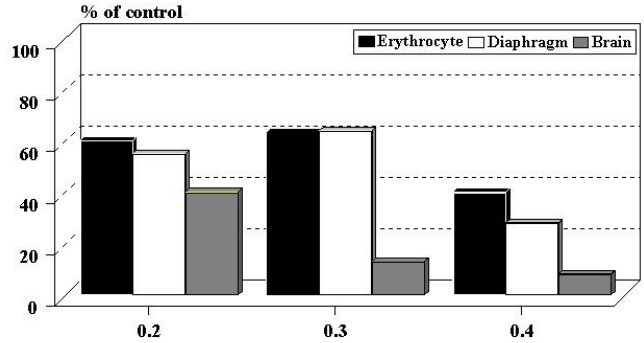

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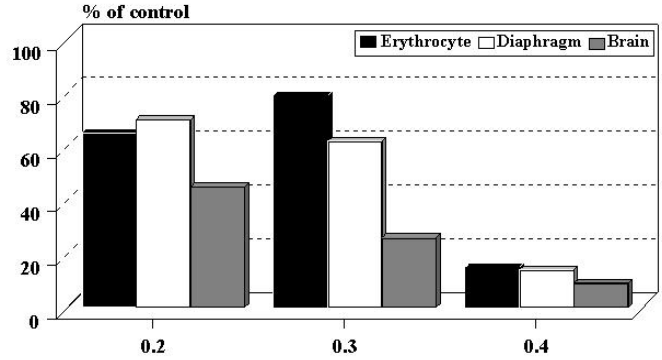

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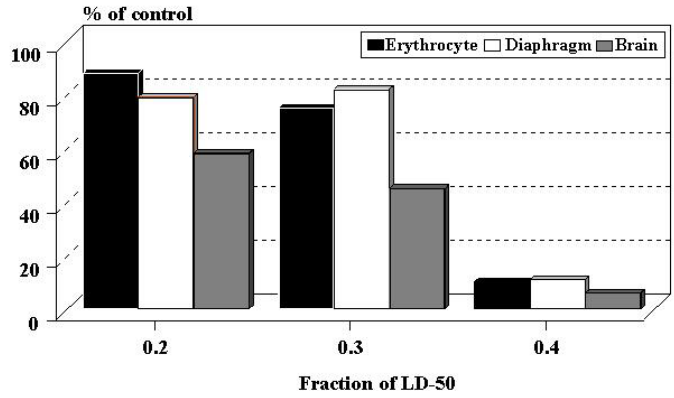

Figure 2. Activities of AChE in erythrocytes, diaphragm and brain in rats treated with tabun 0.2, 0.3 or 0.4 LD_{50} daily.

The three doses of tabun dose-dependently decreased body weight gain during the first two weeks. During the remaining period, body weight gain approached the level in the control group. Tabun in a daily dose of 0.3 LD_{50} decreased body weight gain, food and water consumption especially during the first two weeks of the study. SLA was increased in the same interval and decreased thereafter (Figure 3).

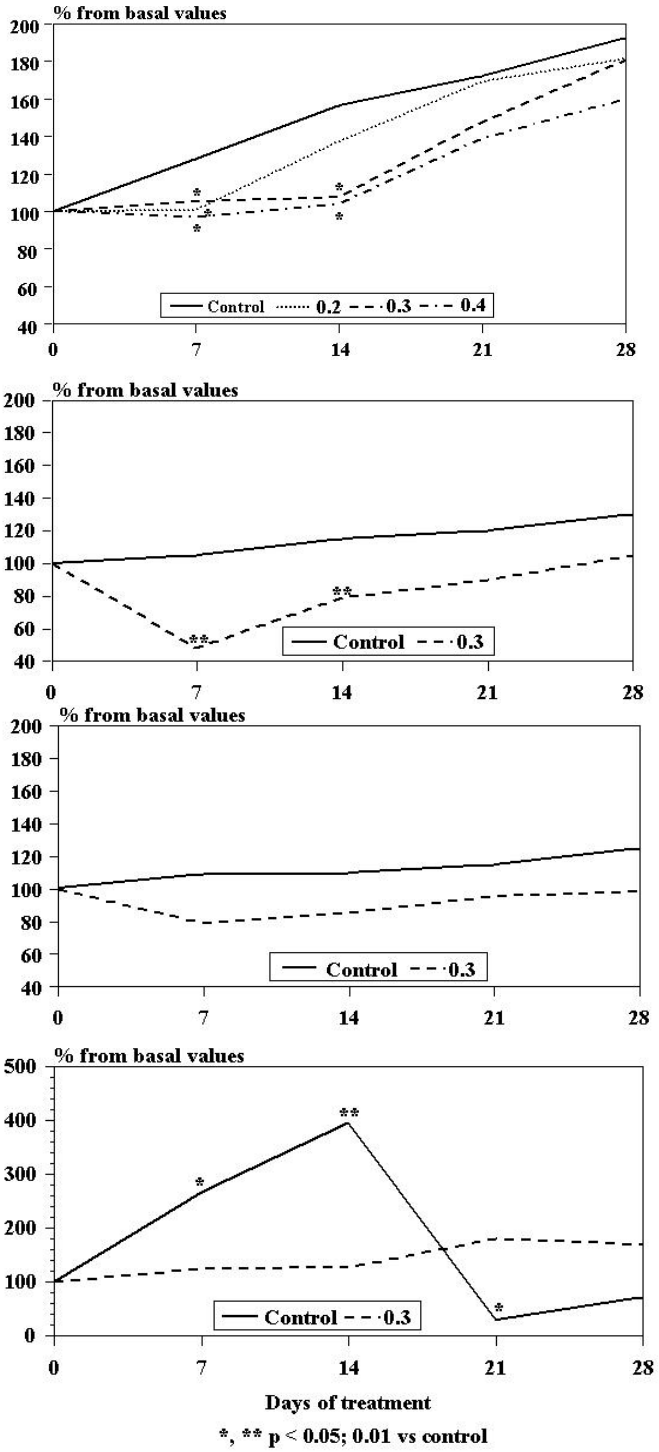

Figure 3. Nutritional and behavioural parameters in rats poisoned sublethally with tabun during 4 weeks.

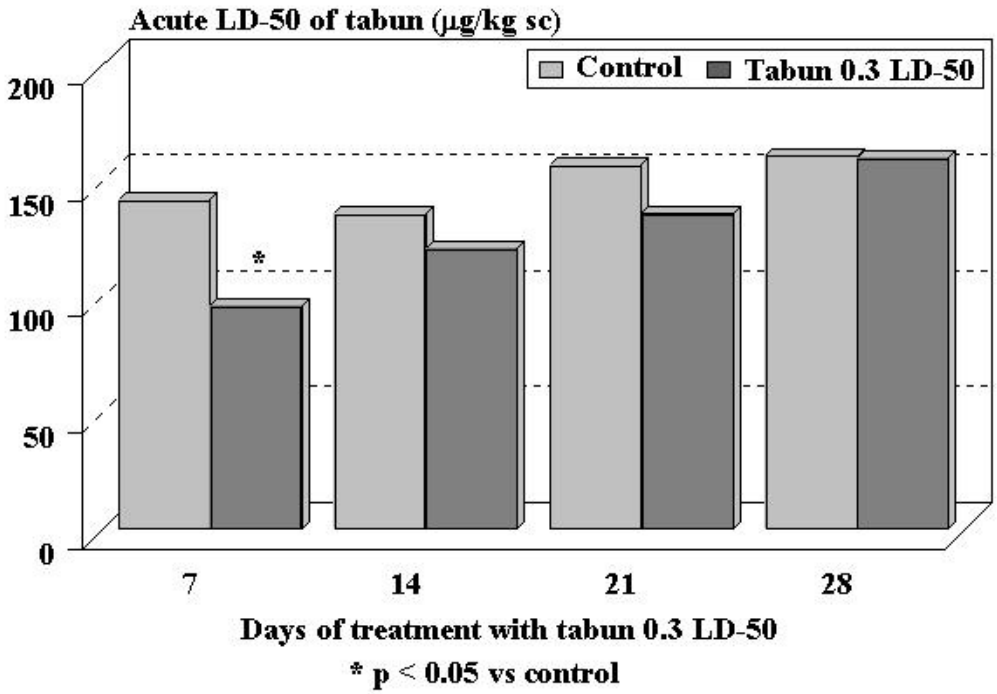

Figure 4. Acute LD_{50} values of tabun in naïve rats and animals treated with tabun 0.3 LD_{50} daily.

Daily treatment of rats with 0.3 LD_{50} of tabun significantly increased acute tabun toxicity only after the first week and gradually approached the control value by the week 4 of the study (Figure 4).

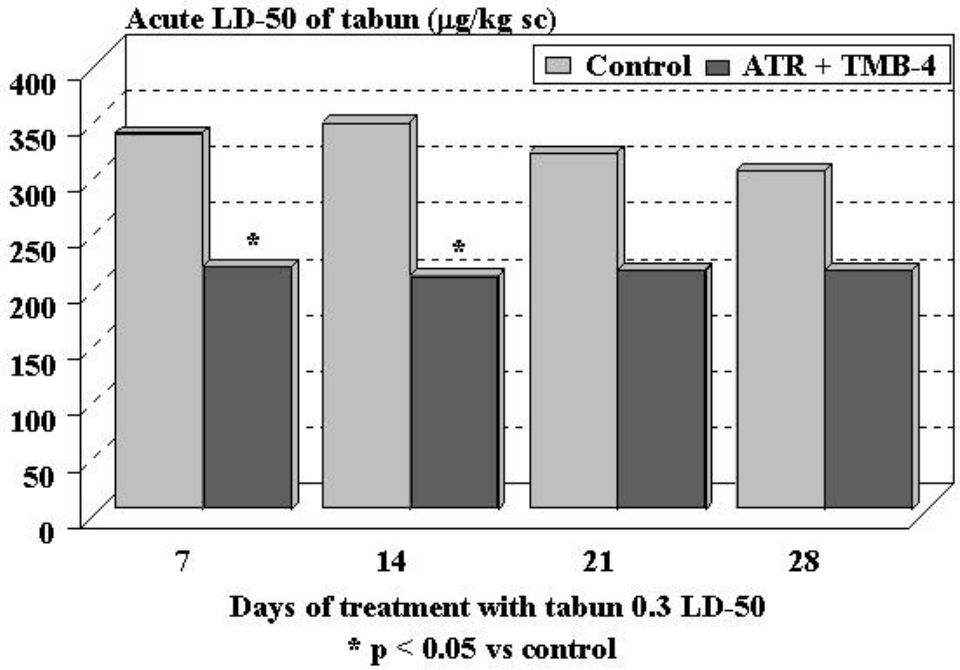

Figure 5. Acute LD_{50} values of tabun in naïve rats and animals treated with tabun 0.3 LD_{50} daily. All the rats received atropine 10 mg/kg and TMB-4 10 mg/kg *im*, immediately after acute tabun administration.

When both the control and the tabun-treated rats were poisoned acutely with tabun and immediately thereafter treated with atropine and TMB-4, the acute tabun LD_{50} values remained constantly around 250 µg/kg, making the difference to the naïve rats significant only after the first two weeks (Figure 5).

DISCUSSION

Tabun was chosen for this study, because it is a very potent irreversible AChE inhibitor and because its equitoxic doses exert the strongest and the fastest tolerance, compared to the ones of sarin or soman (9). This was the reason why the study was set to last for four weeks.

Although some of the rats received in four weeks tabun in a dose that exceeds 11 acute LD_{50}s, which equals more than 8 absolute acute lethal doses of this nerve agent, they survived the experiment. Comparable levels of tolerance - to more than 14 times the acute LD_{50} of nerve agents were obtained by the other authors (9). The results of the present study that lasted for 28 days, along with the similar ones published earlier (9) that lasted for 85 days, clearly indicate that time is a crucial factor for the occurrence of tolerance to OPCs. At the same time, they explain the results obtained in some shorter experiments that lasted for 11 days only (20-22).

Among the general toxic parameters monitored, body weight gain seems to be the most sensitive indicator of the occurrence of tolerance to nerve agents (9). Its decrease always accompanies the sharp decrease in the tissue AChE activities that could be observed during the first two weeks. On the other hand, during the remaining two weeks of the experiment, when tolerance has already occurred, relative plateaus in tissue AChE activities parallels normalisation of the body weight gain and food consumption.

Changes in SLA observed deserve some specific comments. Although it is generally well known that the anticholinesterase agents - both organophosphates and carbamates - decrease the whole-body locomotion (23, 24), our results for the first 14 days of daily treatment with 0.3 LD_{50} of tabun are quite opposite. These at first sight maybe paradoxical results are however in accordance with the ones obtained by D'Mello (19). In his experiment on marmosets a small dose of sarin of 7.5 µg/kg intramuscularly (*im*) increased their whole-body locomotion three times. Moreover, 12.5 µg/kg *im* of sarin returned the number of movement to the control level, while the dose of 17.5 µg/kg *im* decreased it by some 50%. Therefore, nerve agents, administered in smaller doses - up to 0.4 LD_{50} - stimulate SLA, while higher doses have the opposite effect (1). This phenomenon is clearly shown after sarin (25, 26) and soman (27, 28) and repeated dosing with fenitrothion (29) at doses in a range 0.3-0.8 LD_{50}.

Generally speaking, spontaneous regeneration of tabun-inhibited AChE in various tissues is slow. Its regeneration half-times in whole blood, diaphragm and intercostal muscles amount 6.6, 3.6 and 5.9 days, while the same parameter ranges among the brain regions from 7.4 days in basal ganglia to 11.9 days in cortex (30). Notwithstanding these data, it is obvious that in our experiment 0.2 and 0.3, but not 0.4 LD_{50} of tabun daily assured gradual return towards the initial values after weeks 3 and 4 of the study.

Although at least part of the explanation for the occurrence of tolerance to tabun in rats should be found in its metabolism (31), there is no doubt that the other part should rest on the grounds of desensitisation and down-regulation cholinergic and probably some other brain receptors (32).

CONCLUSION

Data on the changes in AChE activity support the assumption that a specific biochemical adaptation to the presence of an organophosphorus cholinesterase inhibitor occurs in the organism of experimental rodents, especially after the week 2 of the experiment. The receptor mechanisms of this adaptation cannot be ruled out, too.

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